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Mechanics of antigen extraction in the B cell synapse

Katelyn M. Spillane[a,b,c] and Pavel Tolar[b,c]

[a]Department of Physics, King's College London, London WC2R 2LS, UK
[b]Immune Receptor Activation Laboratory, The Francis Crick Institute, London NW1 1AT, UK
[c]Division of Immunology and Inflammation, Imperial College London, London SW7 2AZ, UK

Email addresses: katelyn.spillane@kcl.ac.uk (K. M. Spillane),
pavel.tolar@crick.ac.uk (P. Tolar)

Highlights

- Antibodies are an essential mechanism of protection against infectious disease
- Antibody responses start when a B cell acquires antigen, often from the surfaces of another cells
- B cell antigen acquisition is regulated by immune synapse mechanics
- Quantifying immune synapse mechanics and their influence on B cells is now possible using physical manipulation and nanoscopic mechanosensors.
- Understanding B cell mechanosensitivity will open new avenues for particle-based vaccine design

Abstract

B cell encounter with antigen displayed on antigen-presenting cells leads to B cell immune synapse formation, internalisation of the antigen, and stimulation of antibody responses. The sensitivity with which B cells detect antigen, and the quality and quantity of antigen that B cells acquire, depend upon mechanical properties of the immune synapse including interfacial tension, the strength of intermolecular bonds, and the compliance of the molecules and membranes that participate in antigen presentation. In this review, we discuss our current understanding of how these various physical parameters influence B cell antigen extraction in the immune synapse and how a more comprehensive understanding of B cell mechanics may promote the development of new approaches to stimulate the production of desired antibodies.

Key words: B cell activation; immune synapse; mechanobiology; antigen extraction; antigen presentation

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1. Introduction

The production of potent, high-affinity antibodies is an effective mechanism of protection against infectious disease. Soluble antibodies bind selectively to foreign substances, called antigens, both to neutralise them and to stimulate their destruction by innate immune cells. Immunisation induces the production of antibodies that initially vary dramatically in affinity, with most antibodies in the μM range, but some in the nM range. Over time, the average antibody affinity increases in a process called affinity maturation[1].

Antibody responses are initiated when a B cell binds antigen through the B cell receptor (BCR). The BCR comprises a membrane immunoglobulin and a non-covalently associated Igα/β heterodimer that contains immunoreceptor tyrosine activation motifs (ITAMs)[2,3]. Specific binding between the BCR and antigen induces rapid phosphorylation of ITAMs by Src-family kinases and the recruitment of intracellular signalling molecules and adaptors into a signalling complex called the signalosome[4-6]. Signalling leads to transcriptional changes and internalisation of BCR-bound antigen into endosomes, where internalised antigens are processed into peptides, and the peptides complexed with major histocompatibility class II (MHC) molecules. Peptide MHC complexes are transported to the

cell surface and presented to T helper cells. T cells provide signals required for B cell survival and full activation[7,8]. T cell-dependent selection of B cell clones is particularly relevant in the germinal centre (GC), where B cells undergo iterative rounds of somatic hypermutation of the variable region of the immunoglobulin genes and affinity-based selection based upon competition between B cell clones for T cell help[9-12]. B cells that improve their BCR affinity for antigen can leave the GC and differentiate into either memory B cells or plasma cells that produce high-affinity antibodies with the same binding specificity as the BCR. B cell clones expressing high-affinity BCRs internalise and present more antigen, and thus receive more T cell help, than low-affinity clones do[13]. Thus, the quality of binding between the BCR and antigen drives the selection of somatic mutations that improve BCR affinity for antigen over time.

Antigens are collected and concentrated in secondary lymphoid organs such as the lymph node, which has a highly organised microarchitecture that compartmentalises interactions between B cells and other cells of the immune system. This compartmentalisation enables B cells to rapidly probe their environment and maximises the likelihood that a B cell will encounter a rare cognate antigen. Small soluble antigens can diffuse directly from the lymph into the follicle to stimulate B cells[14,15], although in vivo B cells are more likely to be activated by antigens that are displayed on the surfaces of antigen-presenting cells (APCs) including subcapsular sinus macrophages, follicular dendritic cells, and dendritic cells. BCR engagement of membrane antigen triggers a cellular response that results in formation of an immune synapse between the B cell and the APC. The immune synapse is a highly organised structure in which the spatial position of membrane receptors and their signalling activation is tightly regulated to promote optimal B cell responses.

Decades of research have unravelled biochemical signalling cascades that are activated following BCR binding to antigen, but in recent years it has become clear that mechanical communication in the immune synapse also plays an important role. The formation of the B cell synapse is a mechanical process that involves actin-dependent

spreading and contraction of the B cell membrane over the antigen-coated surface[16]. A functional outcome of this membrane restructuring is the binding and collection of antigen, which B cells extract from the antigen-presenting surface using cytoskeletal contractile forces[17]. The exertion of forces has several important implications for B cell responses, most notably the mechanical testing of BCR-antigen bond strength to promote preferential internalisation of high-affinity antigens over low-affinity antigens from the APC[17,18]. Antigens are tethered to APC surface receptors via a series of noncovalent protein-protein interactions that are also sensitive to force. The mechanical stability of these interactions, as well as mechanical properties of the APC membrane, influence the quality and quantity of antigen that B cells internalise. Predicting how individual B cell clones will respond to antigens in vivo will require understanding how these mechanical properties collectively regulate B cell antigen acquisition from the immune synapse.

In this review, we discuss how B cell synapse formation is coordinated within the lymph node and how the molecular components that link antigens to both the B cell and the APC mechanically regulate B cell responses to antigen. Cellular forces are generated by the actomyosin cytoskeleton and the molecular components of the cytoskeletal machinery and dynamics of actin-driven immune synapse formation have recently been reviewed in detail[19]. Here, we will examine how forces in the immune synapse influence the lifetime of molecular bonds involving the BCR, antigen, complexing antibodies, complement fragments, and membrane receptors that display antigens on the APC surface. We also will analyse how engagement of integrins with their ligands in the immune synapse can influence thresholds for B cell sensitivity to antigen binding. We will discuss what is known about each of these molecular components and how they respond to mechanical force.

2. Cells that present antigen to B cells

2.1. Subcapsular sinus macrophages

Opsonised (complement- and/or antibody-coated) antigens are transported in afferent lymphatics to the lymph node, where they are captured by the layer of $CD169^{+}$

macrophages that line the floor of the subcapsular sinus[20-22] (Figure 1). Subcapsular sinus macrophages (SSMs) capture antigens through several surface receptors including complement receptor 3 (CR3; also known as Mac-1, CD11b/CD18, and integrin $\alpha_M\beta_2$) and the Fc receptor for IgG (FcγRIIB). Depletion of either of these receptors does not drastically affect antigen retention, suggesting that these receptors function either redundantly or are used together by the SSM to present opsonised antigen[20,23]. Glycosylated antigens can also be retained by the C-type lectin DC-specific ICAM3-grabbing non-integrin (DC-SIGN)[24]. SSMs capture antigens within minutes of subcutaneous injection[20] and translocate antigens unidirectionally from the subcapsular sinus to the follicle for presentation to B cells. SSMs are poorly endocytic[23,25] and so it is likely that antigens remain on the SSM surface, although it is possible that they internalise antigens into nondegradative compartments[21] and transport antigens into the follicle via transcytosis[26]. Cognate B cells in the follicle slow their migration and form sustained contacts with SSMs displaying opsonised antigens. Contacts between B cells and SSMs may be aided by the SSM expression of the integrin ligands intercellular adhesion molecules 1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), which bind the B cell integrins leukocyte function-associated antigen-1 (LFA-1) (CD11a/CD18, $\alpha_L\beta_2$) and very late antigen-4 (VLA-4) (CD49d/CD29, $\alpha_4\beta_1$), respectively, and facilitate antigen acquisition by cognate B cells[20,22]. Cognate B cells that acquire antigen migrate to the boundary between the B cell and T cell zones to receive T cell help[22,27,28].

2.2. Follicular dendritic cells

Noncognate B cells also can capture complement-coated antigens from SSMs in a transfer process from CR3 on SSMs to complement receptors 1 (CR1/CD35) and 2 (CR2/CD21) on B cells[21,22]. CR2 and CR3 can concomitantly bind to distinct sites on complement fragment C3d to form a stable CR3-C3d-CR2 complex to enable cell-to-cell transfer of complement-coated antigen that does not require the BCR[29]. B cells that acquire antigen migrate into the follicle and deposit the antigen onto CR1/2 expressed by

FDCs. FDCs express considerably higher levels of CR1/2 than B cells do[30], which likely enables them to compete successfully with B cells for complement-coated antigen. FDCs retain antigens on CR1/2 for long periods of time[31] by internalising antigens in an actin-dependent mechanism into nondegradative compartments and periodically recycling them to the surface[32]. They present unprocessed antigens via CR1/2 to naïve and pre-GC B cells[33,34], and mice with FDCs lacking CR1/2 expression have compromised antibody responses[30]. Two-photon imaging experiments of explanted lymph nodes showed that B cells can acquire fluorescent antigens from FDCs via brief (median ~6.5 min) cell-cell contacts[35]. The same experiments demonstrated that naïve B cells could access antigens on FDCs nine days following immunisation, by which time germinal centres had formed[35]. Thus, naïve B cells can access antigen displayed by FDCs in GCs, in support of evidence that naïve B cells have continued access to GC light zones[36]. GCs form approximately one week following immunisation or infection and last for many weeks, suggesting that FDCs continue to make antigen available to B cells during that time[37]. Long-term antigen display may be aided by delivery of antigen to FDCs by noncognate B cells that migrate frequently into ongoing GCs[36].

2.3. Dendritic cells

Dendritic cells are a third APC located in the lymph node. DCs are particularly important for presenting peptide MHC to naïve T cells[38], but they also present unprocessed antigen to B cells to stimulate their activation[39-41]. Resident DCs in the T cell zone can sample soluble antigens from conduits and present them to T or B cells[42]. DCs can retain unprocessed antibody-complexed antigens by capturing them on FcγRIIB, internalising them into non-degradative compartments, and periodically recycling them to the cell surface[38,43]. DC-SIGN performs a similar function for viral antigens such as HIV-1 virions by retaining them in non-lysosomal compartments for periodic presentation to B cells[44]. DCs can also migrate to draining lymph nodes after acquiring antigens in peripheral tissues[45-47]. DCs preferentially home near HEVs, where they are positioned

close to T cells and FRCs[48]. B cells that enter the lymph node through the HEVs can survey the antigens presented by DCs as they migrate along the FRC network to the follicle[40]. Although most DCs are located at the boundary between the B cell and T cell zones, there is a small population of DCs near the subcapsular sinus and occasionally in the follicles that may also present antigens to B cells[49]. In addition, medullary DCs can migrate into B cell follicles after activation[50].

3. In vitro studies reveal molecular mechanisms of B cell activation

While two-photon imaging of B cells in intact lymph nodes have revealed details about B cell motility and contact times with APCs, these measurements cannot resolve molecular details of B cell interactions with APCs and thus our understanding of how these contacts induce B cell activation is incomplete. Progress has been made with in vitro systems that permit high-resolution imaging of single B cells to resolve the spatial organisation of membrane receptors and observe the interaction of B cells with antigen-coated membrane substrates as a model of B cell interactions with APCs.

3.1. BCR distribution in the resting membrane

Super-resolution fluorescence imaging measurements of ex vivo primary B cells have revealed that BCRs reside in the plasma membrane of resting B cells in a heterogeneous mixture of monomers and nanoclusters that contain, on average, tens of BCR molecules and have a radius <~60 nm[51-54]. Diffusion of these nanoclusters is impeded by the cortical actin cytoskeleton, which is linked to the membrane by the ezrin-radixin-moesin family of proteins[55]. Whether this spatial organisation of BCRs has a functional purpose is not clear, although pre-clustering of the receptors may increase sensitivity to antigen by promoting more rapid BCR clustering upon antigen recognition.

3.2. Antigen-triggered B cell spreading

Antigen binding induces the rapid (<60 s) merging of BCR nanoclusters in a BCR-intrinsic mechanism independent of signalling that requires interactions between the Cμ4 domain of the BCR[56,57]. High-affinity BCRs oligomerise and adopt a signalling active conformation more rapidly than low-affinity BCRs do[57]. BCR signalling induces rapid and transient dephosphorylation of ezrin-radixin-moesin family proteins and their local detachment from the plasma membrane, which is required for BCR microcluster growth[57-60]. Disruption of the cytoskeleton-membrane link increases BCR mobility[55] and allows BCR oligomers to grow into microclusters that contain hundreds to thousands of BCRs, signalling proteins, and adaptors[61]. Signals emanating from these clusters induce actin-dependent B cell spreading over the APC surface to maximise B cell interactions with antigen[16]. The extent of B cell spreading depends upon antigen affinity and correlates with signalling; B cells can discriminate monovalent antigens over an affinity range of ~10^6-10^{10} M^{-1}, and the lower threshold for activation drops to ~10^4 M^{-1} for multivalent antigens[62],[63]. Antigen mobility also plays a role, with mobile antigens promoting the formation of larger BCR clusters and more robust signalling compared to immobile antigens[64]. Thus, antigen-triggered BCR signalling is regulated by the actin cytoskeleton and influenced by BCR affinity for antigen and the ability of the B cell to cluster antigens in the immune synapse.

3.3. B cell mechanical testing of antigen affinity

After 2-3 minutes of spreading, the B cell membrane begins to contract[16]. During the contraction phase, B cells actively transport the collected antigen molecules toward the centre of the synapse. The amount of antigen collected is proportional to antigen affinity[16]. During the contraction phase, B cells generate myosin IIa contractile forces that pull on BCR clusters. How myosin physically links to the BCR to exert tension is unknown, but the result is that B cells internalise antigens from the presenting membrane[17,65,66]. Mechanical forces rupture bonds between the BCR and low-affinity antigens while promoting

internalisation of high-affinity antigens, leading to efficient discrimination of antigen affinities in the immune synapse.

3.4. B cell antigen internalisation

The morphological changes that culminate in the formation of an immune synapse are a common feature associated with other activated immune cells including $CD8^+$ T cells and natural killer cells[67-72]. Whereas $CD8^+$ T cells and natural killer cells form synapses to deliver proteases and lytic granules to target cells, B cells form synapses to acquire antigen from APCs. The combination of BCR clustering and mechanical forces enables B cells not only to test antigen affinity, but also to internalise antigens in an affinity-dependent manner. Clustering increases the avidity of the interaction and permits load sharing across multiple BCR-antigen bonds. Load sharing enables low- and moderate-affinity antigens to be internalised even when individual bonds would not be able to withstand the pulling forces[73]. Stochastic simulations suggest that for a cluster of parallel bonds subject to a constant force, the lifetime of the cluster increases monotonically with its size[74,75]. For bonds in parallel, the force is distributed equally among existing bonds in the cluster, thus lowering the load on individual bonds and prolonging the cluster lifetime[76]. Imaging of B cells interacting with antigens bound to flexible membrane substrates showed that mechanical forces create unsynchronised membrane invaginations that are associated with the BCR, actin, and myosin IIa[17]. BCRs that engage high-affinity antigens form large clusters that associate with large amounts of actin and myosin IIa and support long-lived membrane invaginations for internalisation. In contrast, BCRs binding low-affinity antigens form small microclusters with short invagination lifetimes, suggesting that BCR bonds with low-affinity antigens rupture under mechanical load before the antigens can be internalised.

Clathrin-coated pits are the primary mechanism for B cell internalisation of antigen[17,77-79]. Experimentally it has been observed that BCR-antigen bond lifetimes of ~20 s are required for the recruitment of Src-family kinases and assembly of clathrin-coated structures, supporting a kinetic proofreading mechanism for B cell activation in which B cells

need to remain engaged for a finite period of time before becoming signalling capable[80]. It has been shown that the BCR can directly activate myosin IIa[81], and rupture of all BCR-antigen bonds in a cluster leads to rapid dissociation of actin filaments and clearance of myosin IIa associated with the cluster. Together, these observations suggest that BCR signalling regulates the timing and intensity of myosin IIa contractility, which may require BCR tension. Indeed, stochastic simulations support an adaptive model of antigen affinity discrimination in which the strength and timing of mechanical forces is coupled to readouts from the BCR[82].

3.5. BCR-antigen bond mechanics

The strength of a bond — i.e., the force at which the bond is most likely to rupture — is a dynamic property that depends upon bond chemistry, molecular compliance, and loading speed[76,83]. BCR interactions with antigens are noncovalent bonds that form through weak interactions such as Van der Waals forces, hydrogen bonds, hydrophobic interactions, and electrostatic forces, and have energies on the order of k_BT. In solution at equilibrium, molecules continuously associate and dissociate under zero force as the result of thermal fluctuations. The strength of binding between an antibody and an antigen is typically reported as the equilibrium dissociation constant (K_d), which is the ratio of the bond dissociation and association rates at equilibrium in solution and has units of M ($K_d = k_{off}/k_{on}$). The dissociation constant is inversely related to bond affinity ($K_a = 1/K_d$).

Bonds between membrane receptors and their ligands in the immune synapse are subject to lateral and tensile forces that influence the lifetimes of those bonds. Single-molecule force measurements of antibody-antigen bonds indicate that the duration of these bonds is shortened under mechanical load[84,85]. Bonds of this type are called slip bonds and their lifetime can be described according to the function[86]:

$$t_F = t_0 \times \exp\left(\frac{x_b F}{k_B T}\right) \qquad (1)$$

where t_F is the bond lifetime under constant force F, t_0 is the bond lifetime in the absence of force, x_b is the distance the molecular components must be separated for bond rupture (~1 nm for antibody-antigen bonds), k_B is the Boltzmann constant, and T is the absolute temperature (k_BT ~ 4.1 pN nm at 300 K). This bond behaviour has clear implications for B cell discrimination of antigen affinities in the immune synapse. Force is an effective way of testing bond strength on physiologically relevant timescales[87]. An antibody that has a zero-force half-life of 30 minutes in solution will have a half-life of 3 minutes when subject to 10 pN of force, which would permit testing of antigen affinity within the 5-10 minute contact time between a B cell and an APC in vivo[36]. Force also narrows the distribution of bond lifetimes, which helps to improve discrimination of antigens with similar affinities[88]. Importantly, force enables B cells to internalise high-affinity antigens preferentially over low-affinity antigens in the immune synapse[17,66], which is critical for selection of high-affinity B cell clones in the germinal centre and affinity maturation of antibodies[9,11,89].

The force at which a noncovalent bond ruptures depends upon the compliance of the molecules involved. A bond formed between two molecules that are tethered to opposing surfaces and separated at constant speed will rupture at different forces depending upon the stiffness of the molecules, even when the bond chemistry is the same. Bonds formed between molecules of low stiffness are more likely to survive longer, but fail at lower forces, compared to bonds formed between molecules of high stiffness (Figure 2A)[76,83,90]. Molecular stiffness, k, impacts the bond rupture force F as:

$$F = \frac{k_B T}{x_b} \ln \frac{F' x_b}{k_B T k_{off}} \qquad (2)$$

where $F' = kv$ is the bond loading rate (where *v* is the loading velocity) and k_{off} is the equilibrium bond dissociation constant in the absence of force. For a constant loading velocity of 100 nm/s, this model predicts that the rupture force of a molecular bond with a zero-force half-life of 30 min would more than doubled if the molecular stiffness increased from 0.1 to 1,000 pN/nm, which is well within the range of stiffness values measured for adhesion proteins (Figure 2B)[91].

The influence of molecular compliance on the mechanical stability of noncovalent bonds may have a substantial impact on the forces at which BCR-antigen bonds rupture. Mice and humans express five BCR isotypes — IgM, IgD, IgG, IgA, and IgE — and these have different structural elements that may confer different responses to molecular tension. For instance, the IgG and IgD BCRs have highly flexible hinge regions whereas the IgM BCR lacks a hinge region and is instead more rigid. A potential effect of this structural difference was demonstrated in a recent study that revealed different responses to tension by the IgM and IgG BCRs. Measurements using DNA tension sensors showed that the IgM BCR is minimally responsive when tension is <20 pN, moderately responsive to tensions between 20 and 40 pN, and maximally responsive when tension exceeds 50 pN[92]. In contrast, IgG BCRs with identical binding affinity for antigen did not require detectable levels of tension to stimulate B cell activation[92]. Similarly, the IgD BCR has different activation requirements compared to IgM BCR that stem from the flexible IgD hinge region[93]. It is interesting to speculate that the BCR isotypes expressed by different B cell subsets may confer variable sensitivities, and thus trigger different responses, to identical antigens based upon the influence of molecular compliance on the ability of the BCR to transmit force.

3.6. B cell subset-specific mechanics

The spatial organisation of BCRs in the immune synapse also influences mechanical thresholds for B cell activation. Whereas naive B cells form tight contacts with antigen-coated membranes and collect antigens into a large, centralised cluster in the immune synapse, GC B cells engage antigens through lamellipodial-like protrusions and traffic antigens in small clusters to the synapse periphery[65,94]. GC B cells limit the number of antigen-engaged BCRs in each cluster to reduce BCR load sharing and increase the mechanical load on individual bonds. Compared to naïve B cells, GC B cells signal more robustly to activate myosin IIa contractility and exert stronger forces during antigen extraction, leading to more stringent discrimination of antigen affinities[65]. Thus, altered

BCR signalling, receptor spatial organisation, and cytoskeletal activity regulate the different functionalities of naive and GC B cells.

3.7. Immune synapse mechanics

Antigens are tethered to the surfaces of APCs through a series of noncovalent molecular bonds that involve antigen, antigen tethering molecules (i.e., antibodies and complement fragments), and surface receptors (Figure 3A). The surface receptors are transmembrane proteins and, as such, their mechanics are influenced by associations with the actin cytoskeleton and by the compliance of the plasma membrane. Each protein-protein interaction in the chain has a characteristic strength that is influenced by molecular stiffness and externally applied force. Forces applied by the BCR to antigen are transmitted to all bonds in this series and the total force that can be exerted is limited by the stiffness of the chain, which is determined by its softest element. The most probable rupture site is the bond that is weakest under the mechanical load[95].

Recently we demonstrated that the bond rupture site — and the quality and quantity of antigen internalised — could be tuned by modulating the chain stiffness and mechanical stability of the antigen-tether bond. We replaced the antigen tethering molecules with DNA duplexes that were designed to unfold under different forces to release antigen and quantified B cell antigen internalisation from stiff and soft membrane substrates[66]. We found that the relative strength of the BCR-antigen and antigen-tether bonds, and the compliance of the antigen-presenting membrane, collectively influenced the amount of antigen that B cells internalised. When antigens were presented on stiff membranes that resisted deformation under tensile load, B cells used strong forces to rupture the antigens from the DNA tethers prior to internalisation. B cells internalised significantly more high-affinity antigen than low-affinity antigen and increasing the strength of the antigen tether led to a drastic reduction in the amount of antigen internalised. Thus, on membranes of high stiffness, strong forces are transmitted to the molecular chain and the bond rupture site is determined by the relative mechanical stability of the BCR-antigen and antigen-tether bonds.

In contrast, antigen tethering strength did not influence internalisation from soft membranes; antigen uptake was equal from both weak and strong tethers. From soft membranes, B cells still internalised more high-affinity antigen than low-affinity antigen, although their ability to discriminate antigen affinities was diminished compared to internalisation from stiff membranes. The reason is that B cells deform and invaginate soft membranes using weak mechanical forces and pinch the antigen and tether from the membrane in a clathrin-dependent trans-endocytic process[17]. Thus, on soft membranes B cells internalise a combination of antigen, antigen tethering proteins, and membrane components[66]. Taken together, the bond stability and molecular stiffness of all elements within the BCR-antigen-tether-APC chain influence the quality and quantity of antigen that B cells acquire from APCs, which may qualitatively influence the peptide MHC repertoire (Figure 3B).

3.8. Integrin mechanosensing

The BCR is not the only membrane receptor that is sensitive to tension. Integrins are well characterised mechanosensors that promote adhesion and adaptive cellular responses based upon extracellular mechanical cues such as matrix rigidity. The major integrin types expressed by lymphocytes are LFA-1 and VLA-4 [96,97]. The ligands for these integrins are ICAM-1/2 and VCAM-1, respectively, and are expressed by APCs[96]. Integrins are important for B cell migration and formation of immune synapses with APCs. Integrin activity is modulated quickly and reversibly by intracellular signalling, which induces integrins to adopt different structural conformations that bind ligands with different affinities. In resting B cells, integrins exist as monomers with a bent conformation and bind their ligands with low affinity[98,99]. Inside-out signalling triggered by antigen stimulation of the BCR or chemokine stimulation of G-protein coupled receptors induces integrins to change their distribution on the cell surface and/or to adopt a conformation that extends ~20 nm from the cell surface and binds ligands with moderate affinity[100-104]. Forces exerted on bonds between integrins and their ligands cause integrins to adopt an extended open conformation that binds ligands with highest affinity[105]. Ligands provide a strong counter-force, resulting in the formation of a catch bond and outside-in integrin signalling that recruits adaptor proteins

to reinforce the association between integrins and the actin cytoskeleton[106,107]. By stabilising adhesions between B cells and APCs, integrins lower the amount of antigen required to trigger BCR signalling and increase B cell sensitivity to BCR tension[92,108,109]. Why integrin engagement enhances BCR sensitivity to antigen is not known, but in T cells binding between LFA-1 and ICAM-1 enables the transmission of higher forces onto bonds between the TCR and peptide MHC[110]. The mechanism underlying B cell mechanosensitivity is not known, but BCR antigen stimulation increases phosphorylation of the mechanosensing protein CasL (lymphocyte-specific Crk-associated substrate). Mechanical force induces unfolding and phosphorylation of CasL, which then activates downstream signalling[111]. Integrin signalling also activates CasL[112], which may further augment BCR sensitivity to antigen affinity and substrate stiffness.

4. Linking biophysical measurements with antigen presentation in vivo

There is growing evidence that B cell activation is regulated by antigen spatial characteristics, antigen tether strength, APC receptor affinities, and APC membrane stiffness. Here we will discuss what is known about these different properties and how they may influence B cell responses in vivo.

4.1. Antigen spatial characteristics

B cells are sensitive to antigen physical characteristics including antigen density[16,56,57] and valency[113,114]. Antigen multivalency arises in several ways. Viral envelopes display antigenic epitopes in rigid arrays with 5-10 nm spacing, a distance that has been shown to be optimal for B cell activation[115]. Systematic studies using synthetic antigen vehicles such as nanoparticles and virus-like particles to induce immune responses have demonstrated that the density and spatial organisation of antigenic epitopes influence BCR clustering, signalling, antigen internalisation, antigen presentation to T helper cells, and antibody production[93,116-119]. Alternatively, antigen molecules can be crosslinked by soluble antibodies to form immune complexes, which can then be coated with complement fragments and bind through multivalent interactions to either FcRs or CRs or both. APCs

also regulate the spatial distribution of antigen molecules on their surface; for example, FDCs multimerise antigens and display them in discrete, periodic clusters on their surface, which may promote cross linking of BCRs to enhance B cell activation[32,120,121].

4.2. Antigen tethering by antibodies

The antibodies that complex antigens and tether them to APC receptors may set a threshold affinity for the BCR to extract and internalise antigen. Early in the immune response, low-affinity (K_d ~ 10 μM) and non-mutated antibodies are generated by extrafollicular plasma cells[122-124], but later in the response antibodies are generated by affinity-matured plasma cells that have exited the germinal centre[125]. Antibodies shield antigenic epitopes from the BCR and must unbind before the BCR can engage. Antibody-antigen bonds exist in a dynamic equilibrium with a characteristic bond lifetime that is determined by the antibody affinity, so as antibodies affinity mature they will remain bound to antigens for longer periods of time. The result is that the effective epitope concentration will continue to decrease. This process promotes antigen engagement by B cell clones with increasingly higher affinity, which drives selection of high-affinity B cell clones in the GC and affinity maturation of antibodies[126,127]. This process may also promote the development of breadth, as B cell clones with different specificities can bind unmasked epitopes on the same antigen[128].

4.3. APC receptors that present antigen

The receptors APCs use to present antigens to B cells, i.e., complement receptors (CR1/2 and CR3) and Fc receptors, perform other cell functions such as phagocytosis. These receptors must be able to bind opsonised particles and trigger actin remodelling to promote particle ingestion. Clustering of receptors promotes ligand binding; whereas CR1/2 and CR3 bind monomeric ligands with low affinity (K_d ~ 10^6 M), clustered receptors bind ligands through multivalent interactions with high avidity (K_d ~ 10^9 M)[129]. Similarly, FcγRs bind IgG in a 1:1 stoichiometry[130,131] with low to moderate affinity (K_d ~ 1 to 100

μM)[132,133] and require multivalent interactions for triggering[134,135]. Receptor mobility is constrained by the actin cytoskeleton in resting cells[136-138] but ligand binding triggers signalling that leads to actin remodelling, increased receptor diffusion, receptor clustering, and finally internalisation of receptor-bound particles[139-142]. The implications of this receptor behaviour are three-fold. Firstly, although the moderate receptor-ligand affinities may provide a threshold affinity for B cell antigen extraction, clustering increases the avidity of binding between receptors and their ligands and APCs may thus regulate B cell activation by modulating receptor clustering. Secondly, APCs exert forces on particles in order to internalise them, and these forces may be transmitted to B cells in the immune synapse. Finally, APCs may sense forces transmitted through the BCR and respond by locally remodelling actin or modulating the mobility, conformation, or spatial organisation of receptor or adhesion molecules. Similar behaviour of mechanical crosstalk between cells has been observed in immune synapses formed by T cells and DCs[143-145].

4.4. APC membrane stiffness

Biophysical measurements of cell membrane stiffness have revealed that different APCs have distinct mechanical properties that may influence B cell responses to antigen. AFM measurements of FDC and DC membrane stiffness showed that FDCs have stiff membranes that resist deformation whereas DCs have flexible membranes that deform under tensile forces. These differences influence the ability of B cells to discriminate low- and high-affinity antigens; on stiff FDCs, B cells exert strong pulling forces and discriminate antigen affinities efficiently, while on soft DCs B cells use weak pulling forces to acquire antigens and affinity discrimination is poor[66]. Interestingly, inflammatory conditions generally increase APC membrane stiffness and contractility, which may help B cells to capture high-affinity antigens selectively during an ongoing immune response[146,147]. During inflammation APCs also increase surface expression of the B cell integrin ligands

ICAM-1 and VCAM-1, which promote B cell-APC adhesion and increase B cell sensitivity to antigen.

5. Conclusions and outlook

There is a growing body of evidence that mechanical forces influence antigen-triggered B cell activation. Mechanical forces regulate the sensitivity with which B cells discriminate antigen affinities for internalisation and presentation to T cells, which ultimately drives the production and selection of antibodies during an immune response. The field of B cell mechanobiology is still in its infancy, however. Our knowledge stems primarily from in vitro studies of B cells interacting with antigen-coated glass, gel, and membrane substrates and is limited essentially to the B cell receptor and a small number of adhesion molecules such as the integrin LFA-1. In vivo, B cells encounter antigens bound to transmembrane receptors on APCs. How lateral and tensile forces in the immune synapse influence the mechanical stability of these intermolecular bonds and ultimately the amount of antigen that B cells internalise is largely unknown. APCs are mechanically responsive and present antigens in many cases through phagocytic receptors that are also sensitive to force. Although we speculate that there is mechanical crosstalk between B cells and APCs during antigen extraction in the immune synapse, further studies are needed to characterise the role that forces play in mediating communication between the two cells.

Understanding how mechanical forces influence B cell antigen extraction will aid the development of new vaccine formulations that promote the activation of B cells with the potential to produce desired antibodies. Antibody responses are sensitive to the size, geometry, lateral mobility, and elasticity of particle-based adjuvants as well as the density, spacing, and identity of antigenic epitopes[148-152]. If we can understand fundamentally how B cells sense and respond to these various physical cues, then we can precisely tune the particle design to elicit desired B cell outcomes during immune responses.

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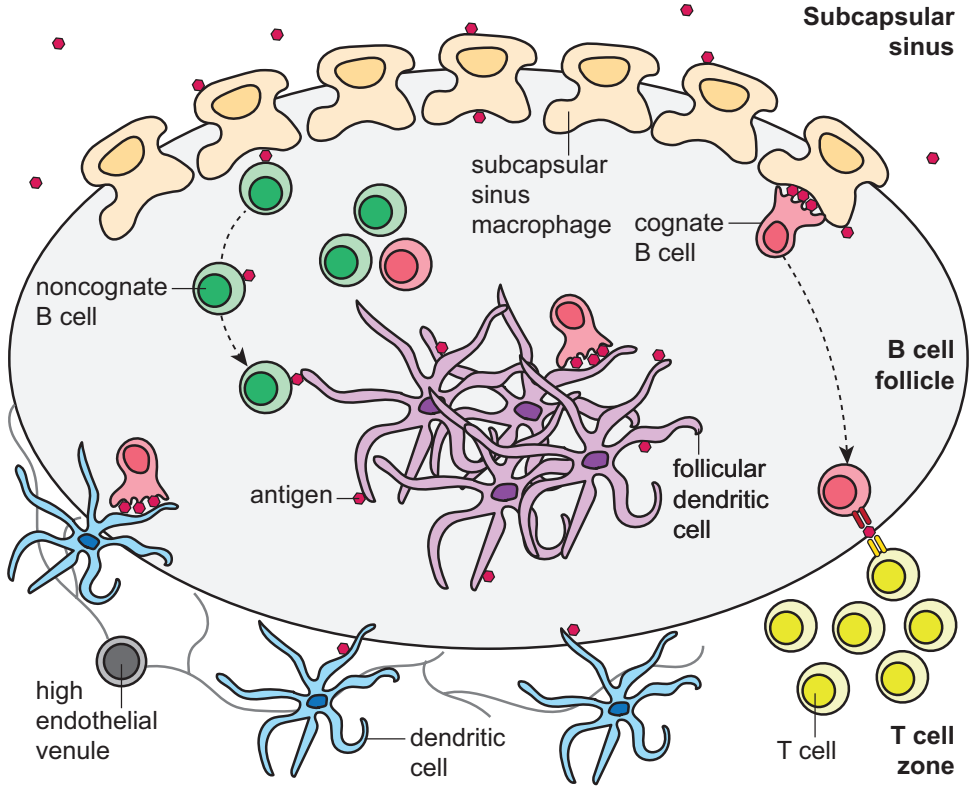

Figure 1. Cellular organisation within the lymph node. Antigens arriving from the lymph can be captured by subcapsular sinus macrophages and transported to the follicle for presentation to B cells. Both cognate and noncognate B cells can capture antigen from SSMs although by different mechanisms. Cognate B cells form sustained cell-cell contacts with SSMs and acquire antigen through the BCR for processing and presentation to helper T cells. Noncognate B cells interact transiently with SSMs and capture antigen through complement receptors, and then migrate into the follicle to deposit the antigens onto FDCs. FDCs retain unprocessed antigens for long periods of time to present antigens both to naïve B cells and to B cells within the germinal centre. Resident and recently migrating dendritic cells can also capture antigens that arrive via conduits and present them to B cells that migrate into the lymph node through the high endothelial venules.

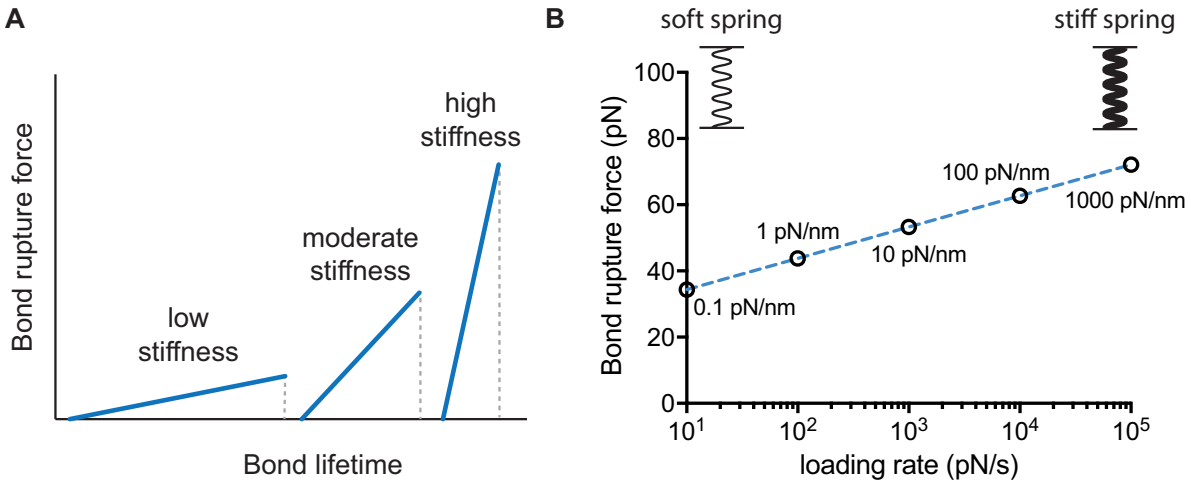

Figure 2. Influence of molecular stiffness on bond rupture. (A) The strength of an intermolecular bond is a dynamic property that depends upon the stiffness of the molecules involved. Bonds formed between molecules of low stiffness are likely to survive for a longer period of time, but rupture at lower forces, compared to bonds formed between molecules of high stiffness. (B) Bond rupture forces can be predicted using the Bell model (Eq. 2) [77,84,87]. For a bond separated at a constant speed of 100 nm/s (unloaded velocity of myosin) [154] that has a zero-force half-life of 30 minutes, the model predicts that increasing molecular stiffness from 0.1 to 1000 pN/nm would cause a ~2.4-fold increase in the bond rupture force.

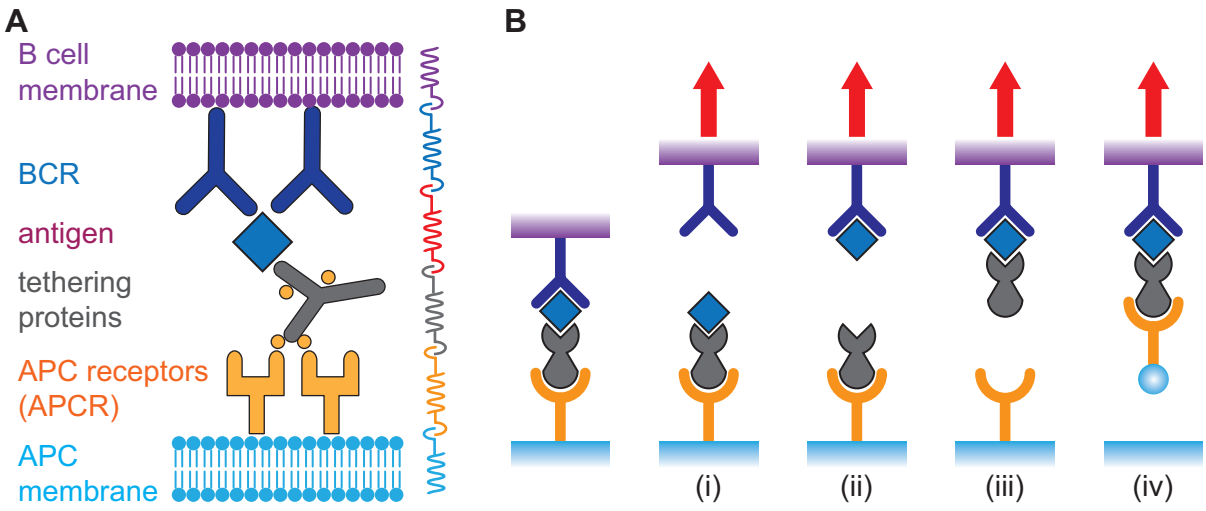

Figure 3. Potential bond rupture sites in the B cell synapse. (A) Antigens are displayed by APCs through chain of molecules that include tethering proteins (antibodies and complement fragments) and antigen receptors. B cells bind antigen through the BCR and apply forces to the bond using actomyosin contractile forces. Forces are transmitted to all molecular bonds within the chain and the weakest bond most probable rupture site. The force at which a bond ruptures depends upon the stiffness of each molecule and the maximum force that can be withstood by each bond, which are represented here as springs and hooks. (B) Where bonds rupture in the immune synapse determines the combination of proteins that B cells internalise and thus the resulting peptide MHC repertoire. In the case of a stiff APC membrane (i-iii), B cells use strong forces to acquire antigen and the bond rupture site is determined by the relative mechanical stability of the BCR-antigen, antigen-tether, and tether-APCR bonds. If the APC membrane is soft (iv), then the B cell can use weak forces to pinch the entire chain of molecules along with a portion of the membrane from the APC.

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